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Pharmacology of a potent long-acting imidazole-5-acrylic acid angiotensin AT_1 receptor antagonist

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Abstract

The angiotensin II antagonistic activity of SB 203220, [*E*-α-[[2-butyl-1-(4-carboxy-1-naphthalenyl)methyl]-1*H*-imidazol-5-yl]-methylene]-2-thiophene-propanic acid], was examined in several *in vitro* and *in vivo* assays. SB 203220 displaced [^{125}I]angiotensin II binding from a variety of tissues including the cloned human AT_1 receptor (IC_{50} 5–15 nM). SB 203220 (10 μM) did not interact with AT_2, endothelin (ET_A and ET_B) or calcitonin gene-related peptide receptors. [^{3}H]SB 203220 bound with high affinity to the AT_1 receptor (K_d = 4.9 nM), but dissociated from the receptor at a much slower rate when compared to [^{3}H]SK&F 108566. SB 203220 antagonized intracellular Ca^{2+} mobilization induced by angiotensin II in rat vascular smooth muscle cells and exhibited a selective and partially insurmountable antagonism of angiotensin II-induced contraction in isolated rabbit aorta. In the aorta, SB 203220 produced a concentration-dependent parallel shift in the concentration-response curve to angiotensin II [$EC_{30} = 5.94 \pm 1.6\ 10^{-11}$ M] and depressed the maximal contractile response to angiotensin II by approximately 35%. The antagonistic effect of SB 203220 in rabbit aorta was slowly reversible compared to SK&F 108566. SB 203220 displayed no agonist activity and had no effect on the contractile responses to KCl, endothelin-1 or norepinephrine. In rats, SB 203220 at 10 mg/kg i.v. inhibited angiotensin II-induced aldosterone release. Intraduodenal or oral administration of SB 203220 (1–10 mg/kg) to conscious rats and dogs inhibited the pressor responses to exogenous angiotensin II. SB 203220 (3–10 mg/kg) also produced a long-lasting (≥ 12 h) antihypertensive response in renin-dependent hypertensive rats and dogs. These data demonstrate that SB 203220 is a potent and highly selective AT_1 receptor antagonist having good oral activity and duration of action in hypertensive models.

Keywords: SB 203220; Angiotensin II; SK&F 108566; Angiotensin AT_1 receptor; (In vitro and in vivo pharmacology)

1. Introduction

Angiotensin II is the primary active component of the renin-angiotensin system and as such exerts a wide variety of physiological actions on the cardiovascular, renal, endocrine, and central nervous systems (Peach, 1977). The biological effects of angiotensin II are mediated through the binding of the peptide to specific cell surface receptors in various target tissues. Based on their differential affinity for nonpeptide receptor antagonists, two major subtypes of angiotensin II receptors (AT_1 and AT_2) have been described (Whitebread et al., 1989; Chiu et al., 1989; Hodges et al., 1992; Timmermans et al., 1993). To date, all known actions of angiotensin II have been shown to be mediated by the activation of the AT_1 receptor subtype. The function of the AT_2 receptor is still unclear. We have previously demonstrated that SK&F 108566 is a selective, potent and orally active nonpeptide AT_1 receptor antagonist (Weinstock et al., 1991; Edwards et al., 1992). As part of an ongoing search to identify novel

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long-acting orally active nonpeptide AT_1 receptor antagonists, we have explored structural analogs of SK&F 108566. Here we report on the *in vitro* and *in vivo* characterization of a naphthyl analog of SK&F 108566, SB 203220, [*E*-α-[[2-butyl-1-(4-carboxy-1-naphthalenyl)methyl]-1*H*-imidazol-5-yl]-methylene]-2-thiophene-propanic acid], a potent selective long-acting imidazole-5-acrylic acid which has a different *in vitro* profile compared to SK&F 108566. The chemical structure, synthesis and preliminary pharmacology of SB 203220 were reported recently (Weinstock et al., 1994).

2. Materials and methods

2.1. Materials

[^{125}I]Angiotensin II [angiotensin II] (2200 Ci/mmol), [^{125}I]Sar^1,Ile^8[angiotensin II] (2200 Ci/mmol), $^{45}CaCl_2$ (14.17 mCi/mg) and an AT_2 receptor binding kit (Drug Discovery Systems: angiotensin II receptor type 2) were purchased from New England Nuclear (USA). Angiotensin II and its analogs were purchased from Sigma Chemical Co. (St. Louis, MO, USA). SB 203220, [^{3}H]SB 203220 (32 Ci/mmol), SK&F 108566, [^{3}H]SK&F 108566, DuP 753 and PD 123319 were synthesized at SmithKline Beecham (King of Prussia, PA, USA).

2.2. Membrane preparation

Plasma membranes were prepared from rat, bovine and human tissues by methods previously described (Edwards et al., 1992). In brief, the tissues were homogenized in 20 mM $NaHCO_3$ for 2×10 s with a Polytron (setting 8). The resulting homogenate was centrifuged at $1000 \times g$ for 10 min at 4°C. The supernatant was centrifuged at $47\,000 \times g$ for 20 min at 4°C and the membranes were washed twice by centrifugation in buffer (50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ and 2 mM Na-EGTA) and finally resuspended in the buffer [2 mg protein/ml]. The recombinant human AT_1 receptor clone was expressed in mouse L cell line (LhAT1D6) as described previously (Aiyar et al., 1994) and was used to characterize SB 203220.

Binding assays were performed by incubating freshly prepared membranes (40–60 μg of protein or $\sim 10^5$ cells per assay) in a final volume of 100 μl of buffer containing 0.025% bovine serum albumin, 300 pM of either [^{125}I]angiotensin II or [^{125}I]Sar^1,Ile^8-angiotensin II in the presence or absence of various concentrations of competing ligands. [^{3}H]SB 203220 binding assay was done using 10 nM radioligand. Incubations were performed at 25°C for 60 min. The reaction was terminated by rapid filtration and filters were counted in a gamma counter or liquid scintillation counter. Nonspecific binding was measured in the presence of 1 μM unlabelled angiotensin II and did not exceed 10% of the total binding. The data were analyzed by computer-assisted nonlinear least square fitting, using the LUNDON software programs (LUNDON Software Programs, Cleveland, OH, USA).

2.3. Effect of SB 203220 on angiotensin II-mediated $^{45}Ca^{2+}$ efflux

Rat thoracic aortic smooth muscle cells were isolated from the thoracic aorta of adult male Sprague-Dawley rats by an explant method and subcultured as described by Ross (1971). Efflux experiments utilizing $^{45}Ca^{2+}$ were performed as described previously (Gleason et al., 1991). The cells were loaded with $^{45}CaCl_2$ by addition of 1 ml of buffer (mM) (118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11 glucose, and 0.03 EGTA, pH 7.4) containing 2 mCi of $^{45}CaCl_2$ and incubated for 120 min at 37°C. The cells were washed, then treated with or without different concentrations SB 203220 before the addition of 10 nM angiotensin II. Efflux curves were generated and the rate constant of $^{45}Ca^{2+}$ efflux was defined as the radioactivity released from the cells per minute at time t divided by the radioactivity remaining in the cells at time t.

2.4. Vascular smooth muscle activity

The effect of SB 203220 on the contractile effects of angiotensin II, norepinephrine, endothelin-1 and KCl were examined in rabbit and rat aorta as we described previously (Edwards et al., 1992). Vascular rings were suspended in organ bath chambers (10 ml) containing Krebs-bicarbonate solution of the following composition (mM): NaCl, 112.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; and dextrose, 11.0. Tissue bath solutions were maintained at 37°C and aerated continuously with 95% O_2/5% CO_2. Cumulative concentration-response curves were constructed by the method of stepwise addition of the agonist according to the technique of Van Rossum (1963). SB 203220 was added to paired tissues 30 min before the initiation of the concentration-response to contractile agonists. Isometric contractions were expressed as a percentage of the response to 60 mM KCl for each tissue. In washout experiments the Krebs-bicarbonate buffer containing SB 203220 was washed out at the indicated times and concentration-response curve to angiotensin II was initiated.

2.5. Inhibition of angiotensin II-induced adrenal aldosterone release

The experimental protocol of Wong et al. (1990a) was followed to study the effect of SB 203220 on

angiotensin II-induced aldosterone release. Rats were treated with vehicle (saline) or SB 203220 (10 mg/kg i.d.). Angiotensin II was then infused (100 ng/kg/min) for 30 min at which time plasma samples were obtained for aldosterone measurements. The aldosterone levels were determined by radioimmunoassay (ICN).

2.6. In vivo activity in conscious rats

Male Sprague-Dawley rats (325–350 g, Charles River Labs, Wilmington, MA, USA) were prepared with indwelling femoral artery, vein and duodenal catheters as previously described in detail (Gellai and Valtin, 1979). Two to three days following surgery rats were placed in a restrainer and mean arterial pressure was continuously monitored via a pressure transducer (Gould). The change in mean arterial pressure in response to bolus i.v. doses of angiotensin II (250 ng/kg) was measured before and at various times after a single dose of SB 203220 (3–10 mg/kg) given intraduodenally.

2.7. Antihypertensive activity in conscious renin-dependent hypertensive rats

Renin-dependent hypertension was established by partial renal artery ligation of one kidney as we have previously described (Ohlstein et al., 1992). Catheters were implanted as described above and mean arterial pressure was measured before, and after the administration of SB 203220. In addition to the vascular catheters, some rats were implanted with a pulsatile Doppler probe (on the ascending aorta) for the measurement of cardiac output, or a bladder cannula for renal clearance studies as previously described in detail (Gellai and Valtin, 1979). Glomerular filtration rate (GFR) was estimated from the renal clearance of inulin (Inutest, Laevosan-Gesellschaft, Linz-Donan, Austria), renal plasma flow (RPF) as the clearance of *p*-aminohippurate, and renal blood flow (RBF) as RPF/(1 − hematocrit).

2.8. In vivo activity in conscious dogs

Male mongrel dogs weighing 9–13 kg were housed under conventional conditions and prepared with a femoral arterial Vascular-Access-Port (VAP) as described in detail previously (Mann et al., 1987). Dogs were allowed at least 3 weeks to recover from surgery prior to study. Dogs were fasted for 18 h with free access to water before being placed in a sling to which they had been trained previously. A catheter was placed in the cephalic vein and the blood pressure response to increasing doses of angiotensin II was monitored before and at various times following either intravenous (0.1 mg/kg) or oral (1 mg/kg) administration of SB 203220. In a separate study, dogs were made acutely hypertensive with an infusion of angiotensin I (100 ng/kg/min). Thirty minutes following establishment of the angiotensin I-induced hypertension, SB 203220 (3 and 10 mg/kg p.o.) was administered and blood pressure monitored for 6 h. To determine the effect of SB 203220 at 13–22 h following administration, drug was administered in the evening to dogs in their home cage. The following morning, 12 h after dosing, animals were placed in the sling and an angiotensin I infusion was initiated and blood pressure monitored for the following 10 h.

2.9. Antihypertensive activity in conscious renin-dependent hypertensive dogs

Renin-dependent hypertension was established in dogs using methods described in detail previously (Brooks and Fredrickson, 1992). Briefly, dogs were prepared as described above and 3 weeks following instrumentation with the Vascular-Access-Port (VAP), an ameroid constrictor was placed on the left renal artery. Two to four weeks following application of the constrictor, blood pressure was significantly increased and the effect of SB 203220 (10 mg/kg p.o.) was evaluated.

2.10. Data analyses

All data are expressed as means ± S.E.M. Statistical analysis was performed by analysis of variance and Dunnett's test for multiple comparisons.

3. Results

Specific [^{125}I]angiotensin II binding to rat mesenteric artery membranes was inhibited by SB 203220 in a monophasic manner with a K_i value of 11.8 ± 2.1 nM (Table 1). SB 203220 exhibited similar high affinity for AT_1 receptor in several other tissues including human AT_1 receptor clone expressed in a stable cell line, LhAT1D6 (Table 1). The K_i values presented in Table

Table 1
Inhibition of [^{125}I]angiotensin II or [^{125}I]angiotensin II (Sar^1,Ile^8) binding by SB 203220 in various membrane or cell preparations

	K_i (nM)
Rat mesenteric artery	11.8 ± 2.1
Rat adrenal cortex	15.1 ± 3.2
Rat aortic smooth muscle cell	9.4 ± 1.5
Human liver	28
Recombinant human AT1 receptor	5.4 ± 1.8
Bovine cerebellum	> 10,000
Bovine ovary	> 10,000

Results are means ± S.E.M. from at least three experiments.

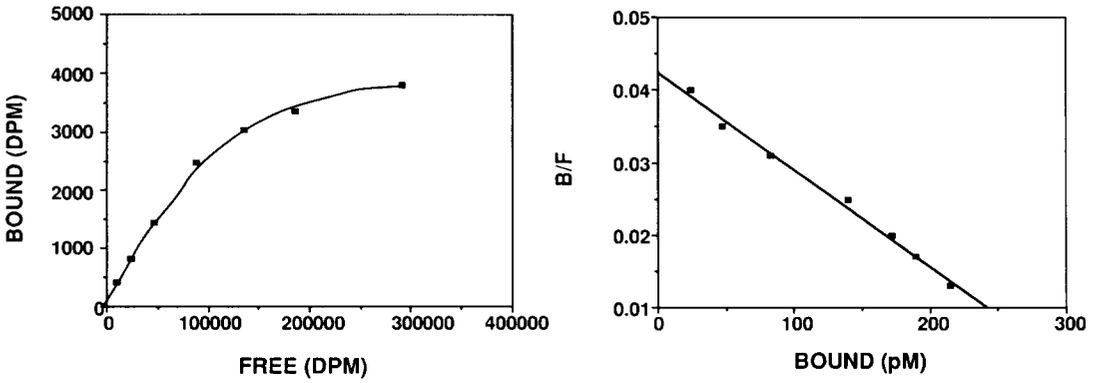

Fig. 1. Scatchard analysis of [^{3}H]SB 203220 binding to bovine adrenal cortex membranes. Angiotensin II (1 μM) was used to define nonspecific binding. Data are representative of three separate experiments performed in duplicate.

1 were obtained in the presence of 0.025% bovine serum albumin. In the absence of albumin, SB 203220 had an 8-fold higher affinity for AT_1 receptor in rat mesenteric artery membranes (K_i 1.5 $\pm$ 0.2 nM). SB 203220 did not displace labelled angiotensin II binding from bovine cerebellum or ovary, which express predominantly AT_2 receptor subtype. The specificity of SB 203220 for angiotensin II receptors was demonstrated by its lack of activity (> 1.0 μM) in other radioligand binding assays such as [^{125}I]endothelin, [^{125}I]calcitonin gene-related peptide, and [^{3}H]arginine vasopressin (data not shown). In experiments designed to study the reversibility of SB 203220 binding to angiotensin II receptors, bovine adrenal cortical membranes were preincubated with 10 nM SB 203220 or SK&F 108566 for 30 min and then extensively washed and total binding with [^{125}I]angiotensin II was estimated. In membranes preincubated with SB 203220, the total binding after washing was decreased by 71%, while preincubation with SK&F 108566 followed by washing had no appreciable effect on [^{125}I]angiotensin II binding. These results suggest that SB 203220 slowly dissociates from the angiotensin II receptor, unlike SK&F 108566 which dissociates rapidly.

[^{3}H]SB 203220 binding to bovine adrenal cortical membranes was specific (80%) and saturable. Scatchard analysis of the saturation binding data was consistent with a single binding site and yielded a dissociation constant (K_d) of 4.9 $\pm$ 1.2 nM and maximal binding capacity (B_{max}) of 454 $\pm$ 18 fmol/mg (Fig. 1). The time course of association of [^{3}H]SB 203220 to bovine adrenal cortical membranes at 30°C was rapid and reached steady state by 30 min and was maintained up to 120 min (Fig. 2). Dissociation experiments were

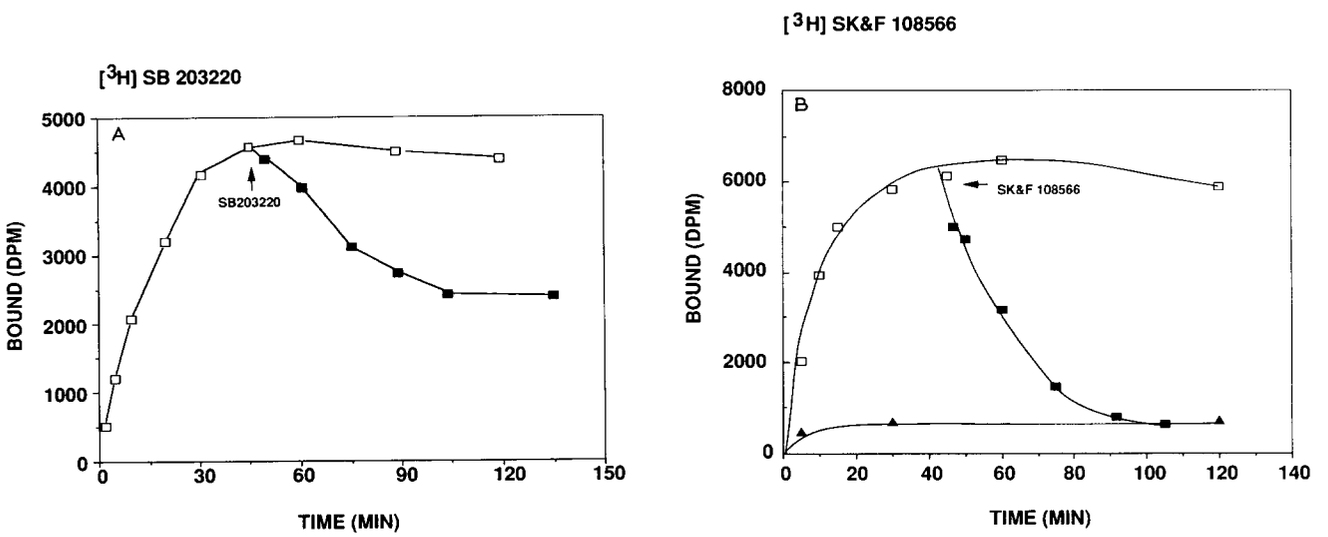

Fig. 2. Association and dissociation of [^{3}H]SB 203220 (left panel) or [^{3}H]SK&F 108566 (right panel) to bovine adrenal cortex membranes. Dissociation was initiated by addition of angiotensin II or unlabelled SB 203220 to [^{3}H]SB 203220 or SK&F 108566 to [^{3}H]SK&F 108566.

Table 2
Displacement of specific [^{3}H]SB 203220 binding to bovine adrenal cortex by various angiotensin II agonists and/or antagonists

	K_i (nM)
Angiotensin I	1440 ± 120
Angiotensin II	6.32 ± 1.8
Angiotensin III	55.5 ± 8.2
[Sar^1,Ile^8]Angiotensin II	7.1 ± 2.1
[Sar^1]Angiotensin II	5.2 ± 1.2
SK&F 108566	6.8 ± 1.8
SB 203220	1.35 ± 0.3
DuP 753	3.5 ± 0.8
PD123319	> 10,000

Results are means ± S.E.M. from at least three experiments.

initiated by addition of unlabeled SB 203220 or angiotensin II (each at 1 μM) to the incubation mixture 45 min after the start of incubation. Only 45% of the bound radioactivity was dissociable by 90 min after the addition of excess unlabelled ligands (Fig. 2, left panel). Comparison of the dissociation rates of [^{3}H]SB 203220 and [^{3}H]SK&F 108566 (Fig. 2, right panel) at 30°C revealed that SB 203220 dissociated from its receptor at much slower rate than did SK&F 108566 which dissociated completely by 90 min. A series of angiotensin II analogs and nonpeptide angiotensin II receptor antagonists were used to compete for [^{3}H]SB 203220 binding to membranes prepared from bovine adrenal cortex and the calculated K_i values from the displacement curves are given in Table 2. The order of potency for various compounds was typical of the AT_1 subtype of angiotensin II receptors. PD 123319, an AT_2-selective antagonist, did not displace [^{3}H]SB 203220 binding from bovine adrenal cortex membranes (Table 2). It was also observed that in the membranes from bovine cerebellum, a tissue rich in AT_2 subtype of angiotensin II receptor, [^{3}H]SB 203220 displayed < 5% specific binding (data not shown).

3.1. Ca^{2+} mobilization

Rat aortic smooth muscle cells were used to characterize the effect of SB 203220 on angiotensin II induced Ca^{2+} mobilization. Fig. 3 (left panel) depicts the time course of 10 nM angiotensin II-mediated $^{45}Ca^{2+}$ efflux and the effect of various concentrations of SB 203220 on angiotensin II response in rat aortic smooth muscle cells preloaded with $^{45}Ca^{2+}$. Immediately following angiotensin II exposure, there was a rapid increase in the rate of $^{45}Ca^{2+}$ efflux from cells which reached a peak within 2 min; thereafter the efflux rate diminished rapidly to basal level (Fig. 3, left panel). Angiotensin II stimulated $^{45}Ca^{2+}$ efflux in a dose-dependent manner with half-maximal effective concentration of 0.8 ± 0.15 nM (data not shown). SB 203220 (0.1 nM to 1μM) alone had no effect on basal Ca^{2+} efflux from the cells preloaded with $^{45}Ca^{2+}$. However, SB 203220 inhibited angiotensin II-induced $^{45}Ca^{2+}$ efflux in a concentration-dependent manner with an observed IC_{50} value of 2 nM ± 0.45 nM, as shown in Fig. 3 (right panel).

3.2. Inhibition of angiotensin II-induced contraction of isolated blood vessels

SB 203220 (0.1–10 nM) antagonized the angiotensin II-induced contractions in isolated rabbit aorta (Fig. 4). The effect of SB 203220 was characterized by a shift to the right of the angiotensin II concentration-response curve as well as a reduction in the maximal contraction (approximately 30%). However, unlike classical noncompetitive receptor antagonists, depression of the

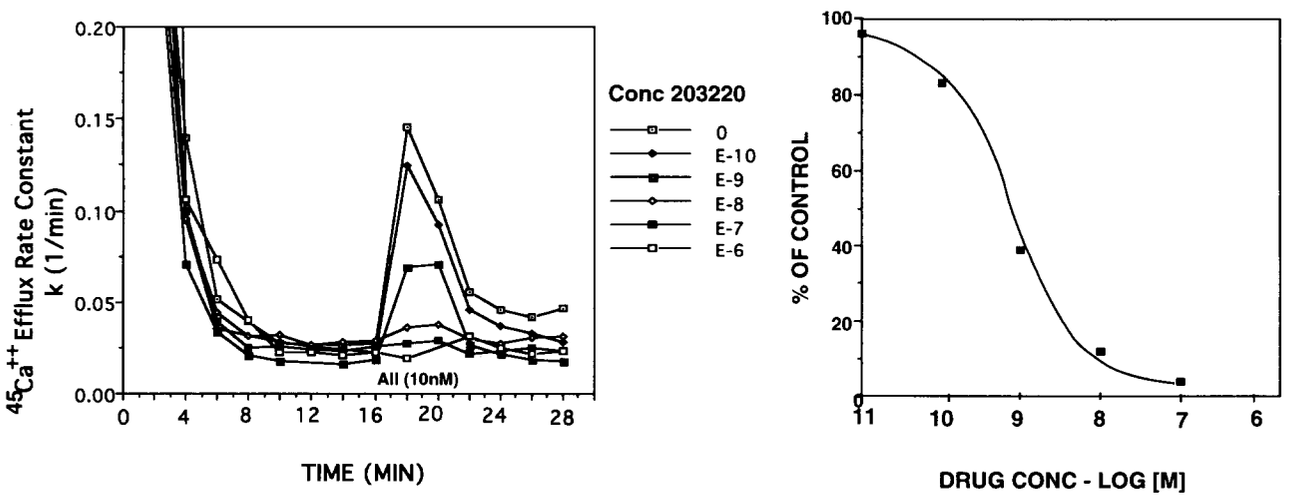

Fig. 3. Left panel: Effect of various concentrations of SB 203220 on 10 nM angiotensin II-mediated $^{45}Ca^{2+}$ efflux in rat aortic smooth muscle cells. Right panel: Concentration-response curve of SB 203220 on angiotensin II-mediated calcium efflux in smooth muscle cells. 100% represents the rate constant of calcium efflux calculated in the presence of 10 nM angiotensin II. Data are representative of three separate experiments performed in duplicate.

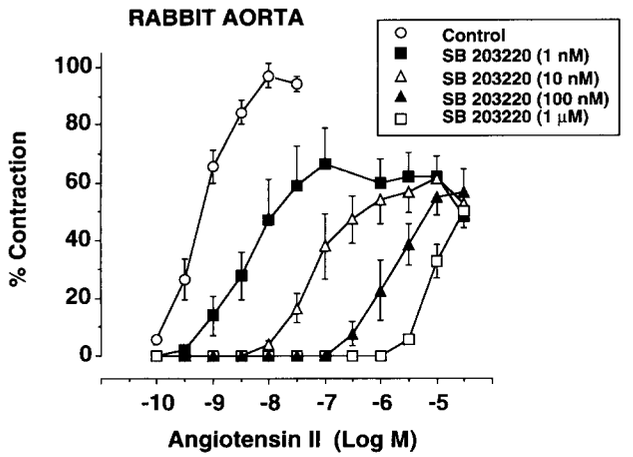

Fig. 4. Concentration-response curves for the effect of SB 203220 on angiotensin II-induced contraction in rabbit isolated aortic rings. Results are expressed as the percentage of the response to 60 mM KCl and are the mean ± S.E.M. of 5 experiments.

maximal contractile response remained constant with increasing concentrations of SB 203220. Despite the reduced maximal contractile response, an apparent dissociation constant (K_{bapp}) of 0.59 ± 0.16 nM was calculated for SB 203220 at the EC_{30} response. Schild analysis of these data yielded a slope of the regression line of 1.17 which is consistent with competitive antagonism. However, in view of the depressed maximal contractile response, the calculation of the absolute value for K_{bapp} and the Schild analysis are not completely valid. The K_b value was similar to the value reported for SK&F 108566 (Edwards et al., 1992). SB 203220 at 10 μM did not significantly modify the contractile response curves to endothelin, norepinephrine or KCl (data not shown).

The reversibility of the antagonist effects of SB 203220 on the contractile effect of angiotensin II is shown in Fig. 5. The antagonist effect of SB 203220 on angiotensin II-induced contraction of rabbit aorta washed out slower than the classically competitive angiotensin II receptor antagonist, SK&F 108566 (Fig. 5). Following washout of SB 203220, the concentration-response curves to angiotensin II returned to control levels in a time-dependent manner. At 180 min following washout of SB 203220, concentration-response curves were still shifted to the right by approximately 2-fold; however, the maximal contractile response had returned to control responses (Fig. 5, top panel). Both the affinity and efficacy components of the concentration-response curves returned to control levels following washout of SB 203220, and the rate of return for each of these components appeared similar. In contrast, SK&F 108566 washed out rapidly from isolated aortic tissue (Fig. 5, bottom panel). Thirty minutes following washout of SK&F 108566, the an-

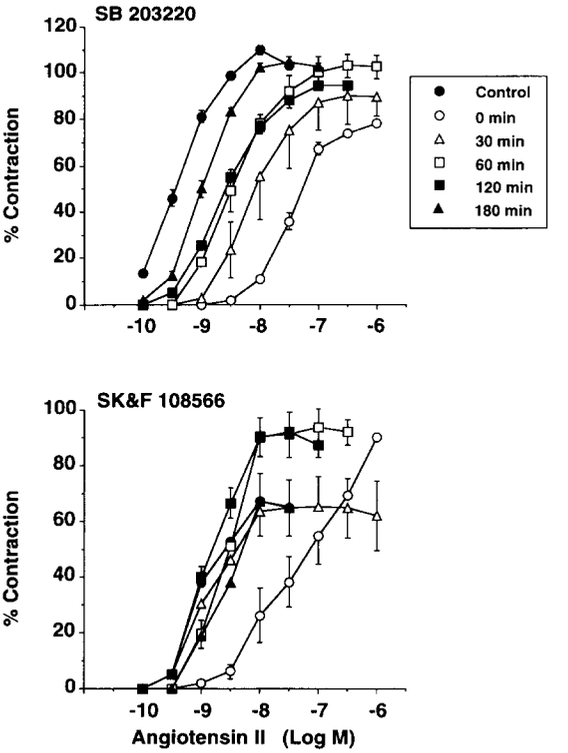

Fig. 5. Reversibility of the effects of SB 203220 (top) and SK&F 108566 (bottom) on the concentration-response curves to angiotensin II. SB 203220 and SK&F 108566 were incubated with the tissues for 30 min. Cumulative concentration-response curves to angiotensin II were then initiated (time 0 min) or the organ baths were rinsed with fresh Krebs buffer containing no antagonist every 30 min. Cumulative concentration-response curves to angiotensin II were performed at the indicated times (30–180 min) following removal of the antagonists. Concentrations of SB 203220 and SK&F 108566 were 10 nM. Results are expressed as the percentage of the response to 60 mM KCl and are the mean ± S.E.M. of 2–3 experiments.

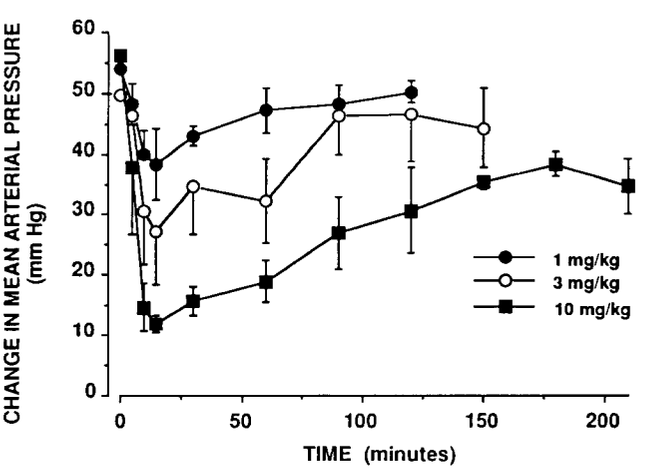

Fig. 6. Effects of various i.d. doses of SB 203220 on the pressor response to angiotensin II (250 ng/kg i.v. bolus). The change in mean arterial pressure to angiotensin II was measured before and at various times after administration of SB 203220 at time 0. Each point is the mean ± S.E.M. of three rats.

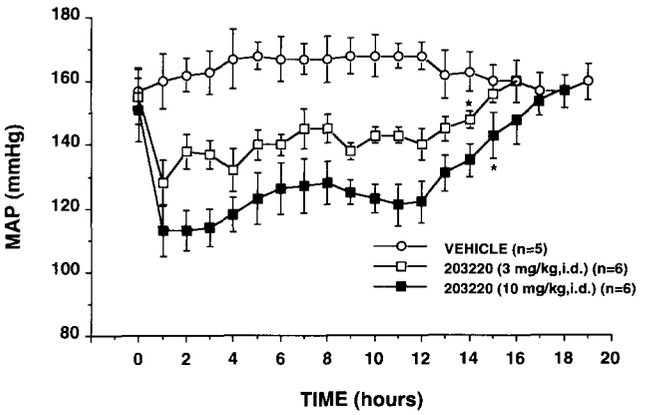

Fig. 7. Time course of the antihypertensive effect of SB 203220 administered intraduodenally in conscious renin-dependent hypertensive rats. * The last time period when values were significantly different from the vehicle-treated group.

giotensin II concentration-response curves were superimposable over the control curves.

3.3. In vivo activity of SB 203220

Angiotensin II increased plasma aldosterone concentration in conscious normotensive rats from 1.4 ± 0.1 to 3.6 ± 0.3 ng/ml ($n = 6$). SB 203220 (10 mg/kg/day) significantly inhibited the rise in plasma aldosterone by 86% (1.7 ± 0.6 ng/ml, $n = 7$).

SB 203220 when given intraduodenally produced a dose-dependent inhibition of the pressor response to angiotensin II in conscious rats (Fig. 6). The inhibitory effect of SB 203220 was rapid in onset (maximum inhibition at 15 minutes post-dose) and at 10 mg/kg i.d. the inhibitory effect lasted for at least 3.5 h. This same dose of SB 203220 (10 mg/kg i.d.) had no effect

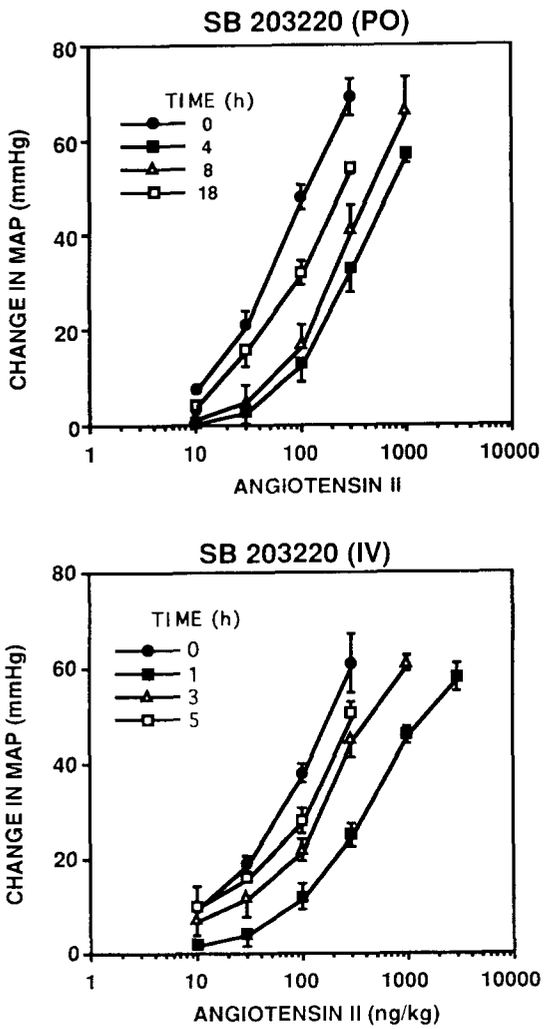

Fig. 8. Time course of the effect of SB 203220 administered orally (1 mg/kg p.o.; upper panel) or intravenously (0.1 mg/kg i.v.; lower panel) on the pressor response curves to angiotensin II in conscious dogs ($n = 4$).

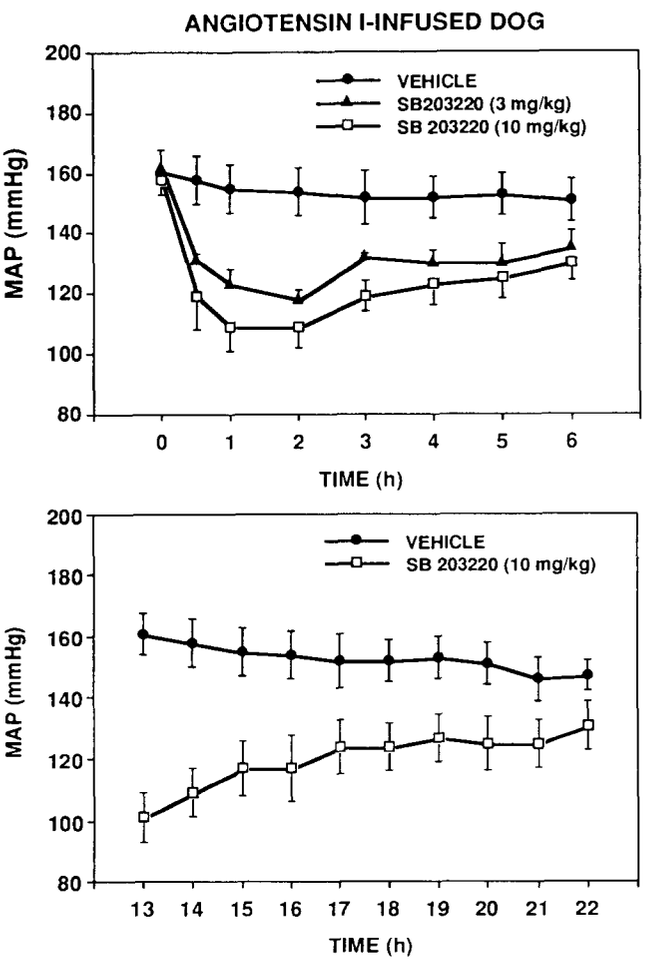

Fig. 9. Duration of the antihypertensive activity of SB 203220 in the angiotensin I-infused dog. Angiotensin I was infused for either 1 h prior and 6 h following administration of drug (upper panel) or between 12 and 22 h following administration of drug (lower panel) ($n = 4$–5 dogs/group).

on equipressor doses of norepinephrine (400 ng/kg i.v.) or vasopressin (100 ng/kg i.v.) (data not shown).

Intraduodenal administration of SB 203220 to renin-dependent hypertensive rats resulted in prolonged antihypertensive activity (Fig. 7). At 3 and 10 mg/kg SB 203220, blood pressure fell approximately 20 and 35%, respectively, and the pharmacological effects of SB 203220 lasted for 14–16 h. SB 203220 had no effect on cardiac output, heart rate or stroke volume, indicating that the effects of SB 203220 on mean arterial pressure were the result of a decrease in total peripheral resistance (Fig. 10). Despite the drop in blood pressure with SB 203220, renal blood flow increased by 28 ± 5% ($n = 6$), while glomerular filtration rate remained unchanged.

In conscious chronically instrumented dogs, angiotensin II resulted in a significant dose-dependent increase in blood pressure, a response that was inhibited significantly by administration of SB 203220 (Fig. 8). SB 203220 administered intravenously at 0.1 mg/kg resulted in a significant rightward shift in the dose-response curve that was greatest at 1 h and had returned towards control values at 5 h post-dosing. Following oral administration of SB 203220, the angiotensin II dose-response curve was significantly shifted to the right for 18 h. In dogs made acutely hypertensive with an infusion of angiotensin I, SB 203220 at 3 and 10 mg/kg p.o. resulted in a dose-dependent reduction in blood pressure (Fig. 9). When SB 203220 was administered 12 h prior to infusion of angiotensin I, residual antihypertensive effects were observed up to 20 h post-dosing (Fig. 9). Constriction of one renal artery with an ameroid constrictor resulted in a significant hypertension with blood pressure increasing to > 150 mm Hg. Subsequent administration of SB 203220 (10 mg/kg p.o.) resulted in a significant antihypertensive response which also lasted at least 12 h (Fig. 10).

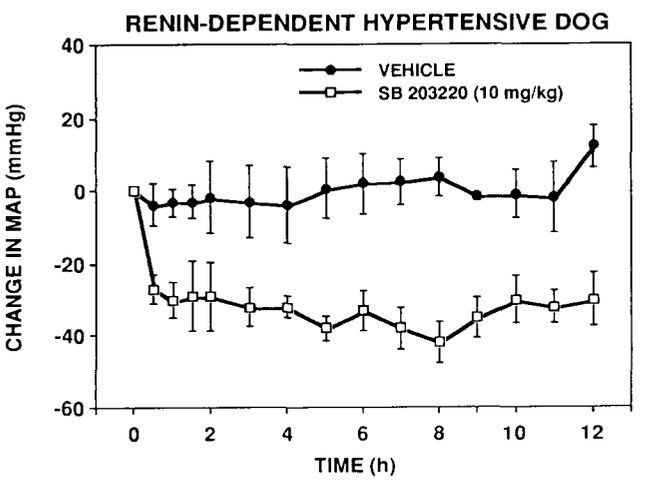

Fig. 10. Time course of the antihypertensive activity of SB 203220 in conscious renin-dependent hypertensive dogs. Baseline mean arterial pressure was 157±5 and 172±6 mm Hg in vehicle ($n = 5$) and SB 203220 ($n = 4$)-treated dogs, respectively.

4. Discussion

In the present study, we have characterized the *in vitro* and *in vivo* pharmacology of SB 203220, a naphthyl analog of SK&F 108566. In binding as well as functional studies, SB 203220 is an angiotensin II receptor antagonist selective for the AT_1 subtype. SB 203220 binding is slowly reversible from the receptor and its effect is partially insurmountable. Furthermore, SB 203220 selectively inhibited the pressor response to angiotensin II *in vivo* and displayed prolonged antihypertensive activity in animal models of renin-dependent hypertension.

SB 203220 selectively inhibited [^{125}I]angiotensin II binding in various tissues with a K_i value of 5–15 nM. The selectivity for the AT_1 receptor subtype was clearly demonstrated by the lack of affinity of SB 203220 for the AT_2 receptor subtype in bovine cerebellum. The affinity of SB 203220 for the AT_1 receptor in rat mesenteric artery membranes was almost 12 times weaker than that of SK&F 108566 and did not correlate well with the rank order for antagonizing angiotensin II-induced contraction in rabbit aorta [K_b 0.59 and 0.21 nM, respectively]. To resolve the discrepancy between the two assays, the possibility of interfering factor(s) was evaluated. It has been shown that bovine serum albumin modulates the binding of non-peptide AT_1 antagonists (Chiu et al., 1991). In the present study it was observed that the apparent binding affinity of SB 203220 was significantly affected by the presence of bovine serum albumin. In the presence of albumin, the K_i value for SB 203220 was 11.8 nM in mesenteric artery, whereas in the absence of albumin, the K_i value was 1.5 nM which compared well with the K_b value (0.59 nM) obtained in fuctional studies using rabbit aorta. Similar studies with SK&F 108566 indicated that the binding was not significantly affected by bovine serum albumin (K_i 1.02 ± 0.04 nM in the absence of albumin and 1.2 ± 0.2 nM in the presence of 0.025% albumin). Therefore, it appears that under *in vitro* conditions SB 203220 binds to albumin to a significant degree. [^{3}H]SB 203220 binding to bovine adrenal cortical membranes was specific, saturable and slowly dissociable. The apparent dissociation constant (K_d) and binding capacity for [^{3}H]SB 203220 to the bovine adrenal cortex membranes was 4.9 ± 1.2 nM and 454 ± 18 fmol/mg, respectively. The binding profile supports that the ligand specifically binds to the AT_1 subtype.

The angiotensin II antagonistic properties of SB 203220 was demonstrated in several functional assays.

SB 203220 antagonized angiotensin II-induced increase of aldosterone *in vivo*. The compound inhibited angiotensin II-induced Ca^{2+} mobilization in smooth muscle cells with an IC_{50} of 2.0 ± 0.45 nM. While SB 203220 shares many features in common with SK&F 108566, there are some important differences. First, unlike SK&F 108566 which rapidly dissociates from the angiotensin II receptor, SB 203220 shows a slower rate of dissociation. Second, while SK&F 108566 has characteristics of a classical competitive antagonist in rabbit aorta (Edwards et al., 1992), SB 203220 displays an antagonism that is best characterized as being partially insurmountable. Although the rightward shift in the concentration-response curve to angiotensin II by SB 203220 is parallel and therefore competitive in nature, SB 203220 produces a 35% decrease in the maximal contractile response produced by SB 203220 and is slowly reversible when compared to SK&F 108566. The pattern of antagonism exhibited by SB 203220 was similar to those of other AT_1 receptor antagonists such as EXP3174 (Wong et al., 1990b), L158809 (Chang et al., 1992), CV-11974 (Noda et al., 1993), and CGP 48933 (Criscione et al., 1993). In general, the authors of those reports suggested that the compounds displayed pseudo-irreversible antagonism due to slow dissociation of antagonist-receptor complex. The antagonistic activity of SB 203220 may also be explained on this basis.

It is interesting to note that the major difference between these two compounds when administered to animals is the longer pharmacological duration of action of SB 203220 when compared to SK&F 108566 (Brooks et al., 1992). This may be a consequence of the slower rate of dissociation of SB 203220 from the AT_1 receptor observed in the binding studies as well as the slower recovery of SB 203220-induced antagonism demonstrated in the rabbit aorta.

In summary, SB 203220 is a potent nonpeptide angiotensin II receptor antagonist with high selectivity for the AT_1 subtype receptor. In both binding assays and functional assays, the antagonism produced by SB 203220 reverses at a slower rate than does SK&F 108566, which may account for the different pharmacodynamic properties of these compounds observed *in vivo*. This difference occurs despite the close structural similarity of the two compounds. As discussed elsewhere (Weinstock et al., 1994), this may be due to the naphthyl ring having a more orthogonal relationship to the imidazole ring of SB 203220 than the corresponding rings of SK&F 108566, and thus altering to some extent the binding locus of the drug with the receptor.

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References

Aiyar, N., E. Baker, H.-L. Wu, P. Nambi, R.M. Edwards, J.J. Trill, C. Ellis and D.J. Bergsma, 1994, Human AT_1 receptor is a single copy gene: characterization in a stable cell line, Mol. Cell. Biol. 131, 75.

Brooks, D.P. and T.A. Fredrickson, 1992, Use of ameroid constrictors in the development of renin-dependent hypertension in dogs, Lab. Anim. Sci. 42, 67.

Brooks, D.P., T.A. Fredrickson, J. Weinstock, R.R. Ruffolo Jr., R.M. Edwards and M. Gellai, 1992, Antihypertensive activity of the non-peptide angiotensin II receptor antagonist, SK&F 108566, in rats and dogs, Naunyn-Schmied. Arch. Pharmacol. 345, 673.

Chang, R.S.L., P.K.S. Siegle, B.V. Clineschmidt, N.B. Mantlo, P.K. Chakravarty, W.J. Greenlee, A.A. Patchett and V.J. Lotti, 1992, In vitro pharmacology of L-158809, a new highly potent and selective angiotensin II receptor antagonist, J. Pharmacol. Exp. Ther. 262, 133.

Chiu, A.T., W.F. Herblin, D.E. McCall, R.J. Ardecky, D.J. Carini, J.V. Dunica, L.J. Pease, P.C. Wong, R.R. Wexler, A.L. Johnson and P.B.M.W.M. Timmermans, 1989, Identification of angiotensin II receptor subtypes, Biochem. Biophys. Res. Commun. 165, 196.

Chiu, A.T., D.J. Carini, J.V. Dunica, K.M. Leung, D.E. McCall, W.A. Price, P.C. Wong, R.D. Smith, R.R. Wexler and P.B.M.W.M. Timmermans, 1991, DuP 532: a second generation of nonpeptide angiotensin II receptor antagonists, Biochem. Biophys. Res. Commun. 177, 209.

Criscione, L., M. De Gasparo, P. Buhlmayer, S. Whitebread, H. Ramjoue and J.M. Wood, 1993, Pharmacological profile of valsartan: a potent, orally active, nonpeptide angiotensin II AT_1-receptor subtype, Br. J. Pharmacol. 110, 761.

Edwards, R.M., N. Aiyar, E.H. Ohlstein, E.F. Weidley, E. Griffin, M. Ezekiel, R.M. Keenan, R.R. Ruffolo Jr. and J. Weinstock, 1992, Pharmacological characterization of the nonpeptide angiotensin II receptor antagonist, SK&F 108566, J. Pharmacol. Exp. Ther. 260, 175.

Gellai, M. and H. Valtin, 1979, Chronic vascular constriction and measurement of renal function in conscious rats, Kidney Int. 15, 419.

Gleason, M.M., E.C. Griffin, P. Nambi and N. Aiyar, 1991, Endothelin stimulates ^{86}Rb efflux in rat glioma C6-BU-1 cells, Neuropeptides 20, 17.

Hodges, J.C., J.M. Hamby and C.J. Blankey, 1992, Angiotensin II receptor binding inhibitors, Drugs Future 17, 575.

Mann, W.A., M.S. Landi, E. Horner, P. Woodward, S. Campbell and L.B. Kinter, 1987, A simple procedure for direct blood pressure measurements in conscious dogs, Lab. Anim. Sci. 37, 105.

Noda, M., Y. Shibouta, Y. Inada, M. Ojima, T. Wada, T. Sanada, K. Kubo, Y. Kohara, T. Naka and K. Nishikawa, 1993, Inhibition of rabbit aortic angiotensin II (angiotensin II) receptor by CV-11974, a new nonpeptide angiotensin II antagonist, Biochem. Pharmacol. 46, 311.

Ohlstein, E.H., M. Gellai, D.P. Brooks, L. Vickery, J. Jugus, A. Sulpizio, R.R. Ruffolo Jr., J. Weinstock and R.M. Edwards, 1992, The antihypertensive effect of the angiotensin II receptor antagonist DuP 753 may not be due solely to angiotensin II receptor antagonism, J. Pharmacol. Exp. Ther. 262, 595.

Peach, M.J., 1977, Renin-angiotensin system: biochemistry and mechanisms of action, Physiol. Rev. 57, 313.

Ross, R., 1971, The smooth muscle cell II; growth of smooth muscle in culture and formation of elastic fibers, J. Cell. Biol. 50, 172.

Timmermans, P.B.M.W.M., P.C. Wong, A.T. Chiu, W.F. Herblin, P. Benfield, D.J. Carini, R.J. Lee, R.R. Wexler, J.A.M. Saye and R.D. Smith, 1993, Angiotensin II receptors and angiotensin II receptor antagonists, Pharmacol. Rev. 45, 206.

Van Rossum, J.M., 1963, Cumulative dose-response curves. II. Technique formalizing of dose-response curves in isolated organs and the evaluation of drug parameters, Arch. Int. Pharmacodyn. Ther. 143, 299.

Weinstock, J., R.M. Keenan, J. Samanen, J. Hempel, J.A. Finkelstein, R.G. Franz, D.E. Gaitanopoulos, G.R. Girard, J.G. Gleason, D.J. Hill, T.M. Morgan, C.E. Peishoff, N. Aiyar, D.P. Brooks, T.A. Fredrickson, E.H. Ohlstein, R.R. Ruffolo Jr., E.J. Stack, A.C. Sulpizio, E.F. Weidley and R.M. Edwards, 1991, 1-(-carboxybenzyl) imidazole-5-acrylic acids: potent and selective angiotensin II receptor antagonists, J. Med. Chem. 34, 1514.

Weinstock, J., D.T. Hill, R.M. Keenan, R.G. Franz, D.E. Gaitanopoulos, G.R. Girard, D.S. Eggleston, N. Aiyar, E. Griffin, E.H. Ohlstein, D.P. Brooks, M. Gellai, T.A. Fredrickson, E.F. Weidley and R.M. Edwards, 1994, A potent long-acting imidazole-5-acrylic acid angiotensin II AT-1 receptor antagonist, Bioorg. Med. Chem. Lett. 4, 23.

Whitebread, S., M. Mele, B. Kamber and M. De Gasparo, 1989, Preliminary biochemical characterization of two angiotensin II receptor subtypes, Biochem. Biophys. Res. Commun. 163, 284.

Wong, P.C., S.D. Hart, A.M. Zaspel, A.T. Chiu, R.J. Ardecky, R.D. Smith and P.B.M.W.M. Timmermans, 1990a, Functional studies of nonpeptide angiotensin II receptor subtype-specific ligands: DuP 753 (angiotensin II-1) and PD123177 (angiotensin II-2), J. Pharmacol. Exp. Ther. 255, 584.

Wong, P.C., W.A. Price Jr., A.T. Chiu, J.V. Dunica, D.J. Carini, R.R. Wexler, A.L. Johnson and P.B.M.W.M. Timmermans, 1990b, Nonpeptide angiotensin II receptor antagonists. XI. Pharmacology of EXP3174: an active metabolite of DuP 753, an orally active antihypertensive agent, J. Pharmacol. Exp. Ther. 255, 211.